

Gene Sequence Structure

*

211 bp

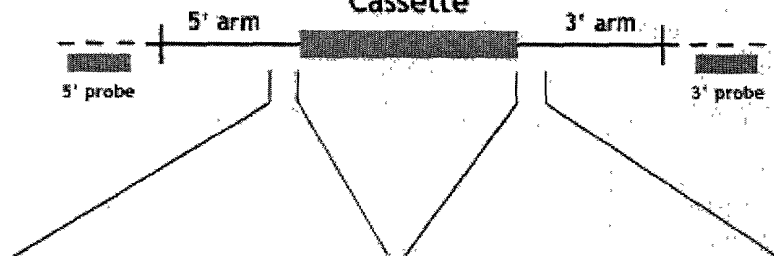
Sequence Deleted

355 bp

Size of full-length
cDNA: 2486 bp

Targeting Vector* (genomic sequence)

LacZ-Neo Cassette



Arm Length:
5': 2.5 kb
3': 1 kb

————— Targeting Vector
- - - - - Endogenous Locus

* Not drawn to scale

5' >CATGCATACATGGGTGCACGT
GCGTGCGTGCAAACACCCACACAC
AGATAGATAAATGTAAAAAATAC
TTAGGCAATGCTGTTTTTTTAAAG
CTTGGGAAATTAATATTTTTGAAC
ACTGCTGATGTGGGATTTTAAGTT
ACAAATGTTAACTTGTTCTCCTT
AGTGACTTTTGGGATGGTCAATCC
ACATATGTACT<3'
(SEQ ID NO:3)

5' >GTATGGCAACAAACAGCTGTA
CAGTGTGAAGAACAGCAGCCGCAT
CTACTACGAGAACGTTCTTCTCGG
CATCCCCAGAGTGCGGCAACTGCC
AGTCCGAAACAACACTTGCAAGGT
CTACCCAGCTTTCCAGTCCCTGGT
CAGCGACTGCTACAGCAAGTACAC
AGTGGAAAACGAAGACTTCTCTGA
TTTTGGCCTCA<3'
(SEQ ID NO:4)

FIGURE 4

GGCAGAACGGGCATAGTGGAACGGACCGTGTGGTATGCTCCGCGATGTCTGAGGCGACTTGGTG
GTACCGAGGAGGGACTTCAAAACATGACCTGCATTACAGAAGGGAAGCGGAGGTTAACACCACA
CTCGAGGAGTTGTTACTCTATTTTTATTTTCTTAATAAATCTATGCATATTGACTTTTGGGATGG
TCAATCCACATATGTACTATTTAAACAAAGTTATGTCTGTCTGTTTGTGGACACTTCTCTACC
TGATGATGAAAGAAGCAGCTTTAGGTCCATTTCGGAGCATAACTGAGTTTTGGAAGTTCATGGAA
GGACCCCTCATCGACGGCTTGTACTGGGACTCGTGGTATGGCACCACAAACAGCTGTACAGTGTGA
AGAACAGCAGCCGCATCTACTACGAGAACGTTCTTCTCGGCATCCCCAGAGTGCGGCAACTGCG
AGTCCGAAACAACACTTGCAAGGTCTACCCAGCTTTCCAGTCCCTGGTCAGCGACTGCTACAGC
AAGTACACAGTGGAAAACGAAGACTTCTCTGATTTTGGCCTCAAACGCAATCCAGAATGGACGC
ACACGCCTTTCTCCCGCACTGCCCCATGGCACTGGGGGTTTGTGGCGTATACCGAGATGGAGG
ATATATAGTCACGTTATCAAAATCAAAATCTGAAACCAAAGCCAAATTTGTTGACCTTCGACTG
AACAACTGGATTAGCAGAGGCACCAGGGCTGTTTTTATTGATTTCTCCCTGTACAATGCTAATG
TCAACCTGTTTTGTCATCATCAGGCTGCTGGCAGAGTTCCCTGCGACGGGTGGGCTCCTCACCTC
CTGGCAGTTCTACTCTGTGAAGCTCCTCAGATACGTCTCCTACTACGACTACTTCATTGCCTCC
TGTGAAGTCATATTTTGTATTTTCTCTTTGTCTTCATAATACAAGAACTGAGGAAAGTGAACG
AGTTTAAGTCTGCCTATTTTCAAGTGTCTGGAAGTGGCTGGAGATGCTGCTCCTGCTGCTCTG
TTTTCTCGCCGTGTCTTCTATGCATACTGTAACATGCAGAGCTTCTCTTGCTTGGACAGCTG
CTGAAAAACACTGACAGCTATCCCGACTTTTACTTCCTTGCATACTGGCACATTTACTATAACA
ACGTAATTGCTATCACTATCTTCTTTGTCATGGATAAAGATATTCAAGTTCATAAGCTTCAATGA
GACAATGTCGCAGCTGTTCATCAACACTCTCCCGCTGCATGAAGGACATCGTGGGGTTCGCCATC
ATGTTCTTCATCATCTTCTCTGCTTATGCCCAGTTGGGATTTCTGGTTTTTGGGTCACAGGTTG
ATGATTTTTCAACTTTTCAAAATTCCATATTTGCACAATTTCAATTTGTCTCGGGGACTTTAA
CTTTGCTGGCATCCAGCAGGCCAACTGGATCTTGGGGCCCATCTACTTCATCACGTTTCATCTTC
TTTGTGTTCTTTGTGCTCCTGAACATGTTCTTGGCAATAATTAATGACACCTATTCTGAAGTTA
AGGCTGATTATTCAATAGGCAGAAGACCAGATTTTGAAGTGGTAAAATAATTCAAAAGAGTTG
CTTTAATGTTCTCGAGAACTCAGACTCAAGAAAGCTCAAGCTAAAGAAGAAAAGAAAATGCAA
ACCACTGACTTGGCCCAGAGAGCCAGAAGAGAAGGCTTTGATGAAAGTGAGATCCAAGAGGCAG
AGCAGATGAAAAGATGGAAGGAAAGGCTTGAAAAAAGTATTATTCTACAGAAATTCAAGACGA
TTATCAGCCTGTCACTCAGCAAGAATTCCGAGAGCTCTTTTTATACGCGGTGGAGCTTGAGAAG
GAATTACACTATGTCAGTTTAAACTGAACCAACTGATGAGAAAGCTGCACTAGCAGGCTGACA
AGTGGAGTCATTTTATAAGAAAAGGCAACCGAAGAAATTCATTTCAGTATGAAGATTTTTTCCTC
TCAATTTTCTTCAGTAATGCAAAAGAAAAACCAAAAAGTAACCAGAAGTGCTTTTTTATTTCAA
GTTCTTGAAGTAAAAGAGTAAAACCTTGTCTTTTGCTAACAGCCGTGTCTGCAGTAAAACAAT
GAAGGAGCCTGCGTGTTCCTAAGTGTGGAGAGGATCTGCGGGAATGTGGAACAGCTTTCCTTG
CCTACTGGAACCACAAACAAGCACACAATGGGACTCTCTGAGTGCCTGACAAAGTGAACGCAAG
TACAGCCAAGCACACATGGTGAAGTGTGAGGGAACACAAGCACTTTATGGCGTCAACTTTCAAG
GAACATATTTTATATGGATTTTGAAGAGTCTTGTGCTGATAAGAACTTCAAGAAGTCTAAGC
TTGGCTTTGATTCTCTTGTATTCTTATATTCTCAAGCACCGGAACACGATCCTCCTTCTGGG
CATTCCTAGGGAAGATAAACTCTGTAAAGCAAAAAAAGAAAAAAGAAAAA (SEQ ID
NO : 1)

FIGURE 1

MSEATWWYRGGTSKHDHLHYRREAENVNTTLEELLLYFIFLINLCILTFGMVNPHEMYLKNKVMSSL
FVDTSLPDDERSFRSIRSITFWKFMEGPLIDGLYWDSWYGTKQLYSVKNSRIYYENVLLGI
PRVRQLRVRNNTCKVYPAFQSLVSDCYSKYTVENEDFSDFLKRNPEWTHTPSSRTAPWHWGFV
GVYRDGGYIVTSLSKSKSETKAKFVDLRLNNWISRGTRAVFIDFSLYNANVNLFCIIRLLAEFPA
TGGLLTSWQFYSVKLLRYVSYDYFIASCEVIFCFLFVFIIQELRKVNEFKSAYFRSVVNWLE
MLLLLLCFLAVSFYAYCNMQSFLLLGQLLKNTDSYPDFYFLAYWHIYYNNVIAITIFFAWIKIF
KFISFNETMSQLSSTLSRCMKDIVGFAIMFFIIIFSAYAQLGFLVFGSQVDDFSTFQNSIFAQFR
IVLGDFNFAGIQQANWILGPIYFITFIFFVFFVLLNMFLAIINDTYSEVKADYSIGRRPDFELG
KIIQKSCFNVLEKLRLKKAQAKEEKKMQTTDLAQRARREGFDESEIQEAEQMKRWKERLEKKYY
STEIQDDYQPVTTQQEFRELFYAVELEKELHYVSLKLNQLMRKLH (SEQ ID NO:2)

FIGURE 2

underlined = deleted in targeting construct

BOLD = sequence flanking Neo insert in targeting construct

GGCAGAACGGGCATAGTGGAACGGACCGTGTGGTATGCTCCGCGATGTCTGAGGCGACTT
GGTGGTACCGAGGAGGGACTTCAAACATGACCTGCATTACAGAAGGGAAGCGGAGGTTA
ACACCACACTCGAGGAGTTGTTACTCTATTTTATTTTCTTAATAAATCTATGCATATTGA
CTTTTGGGATGGTCAATCCACATATGTACTATTTAAACAAAGTTATGTCTCTCTGTTTG
TGGACACTTCTCTACCTGATGATGAAAGAAGCAGCTTTAGGTCCATTCGGAGCATAACTG
AGTTTTGGAAGTTCATGGAAGGACCCCTCATCGACGGCTTGACTGGGACTCGTGGTATG
GCACCAAACAGCTGTACAGTGTGAAGAACAGCAGCCGCATCTACTACGAGAACGTTCTTC
TCGGCATCCCCAGAGTGCGGCAACTGCGAGTCCGAAACAACACTTGCAAGGTCTACCCAG
CTTTCCAGTCCCTGGTCAGCGACTGCTACAGCAAGTACACAGTGGAAAACGAAGACTTCT
CTGATTTTGGCCTCAAACGCAATCCAGAATGGACGCACACGCCTTCTTCCCGCACTGCCC
CATGGCACTGGGGGTTTGTGGCGTATACCGAGATGGAGGATATATAGTCACGTTATCAA
AATCAAATCTGAAACCAAAGCCAAATTTGTTGACCTTCGACTGAACAACTGGATTAGCA
GAGGCACCAGGGCTGTTTTTATTGATTTCTCCCTGTACAATGCTAATGTCAACCTGTTTT
GCATCATCAGGCTGTGGCAGAGTTCCCTGCGACGGGTGGGCTCCTCACCTCCTGGCAGT
TCTACTCTGTGAAGCTCCTCAGATACGTCTCCTACTACGACTACTTCATTGCCTCCTGTG
AAGTCATATTTTGTATTTTCTCTTTGTCTTCATAATACAAGAACTGAGGAAAGTGAACG
AGTTTAAGTCTGCCTATTTTCAAGTGTCTGGAAGTGGCTGGAGATGCTGCTCCTGCTGC
TCTGTTTTCTCGCCGTGTCTTTCTATGCATACTGTAACATGCAGAGCTTTCTCTTGCTTG
GACAGCTGCTGAAAAACACTGACAGCTATCCCGACTTTTACTTCCTTGCACTACTGGCACA
TTTACTATAACAACGTAATTGCTATCACTATCTTCTTTGCATGGATAAAGATATTCAAGT
TCATAAGCTTCAATGAGACAATGTCGCAGCTGTCATCAACACTCTCCCGCTGCATGAAGG
ACATCGTGGGGTTCGCCATCATGTTCTTCATCATCTTCTCTGCTTATGCCCAGTTGGGAT
TTCTGGTTTTTGGGTCACAGGTTGATGATTTTCAACTTTTCAAATTTCCATATTTGCAC
AATTTTCAATTTGCTCCTCGGGGACTTTAACTTTGCTGGCATCCAGCAGGCCAACTGGATCT
TGGGGCCCATCTACTTCATCACGTTTCATCTTCTTTGTGTTCTTTGTGCTCCTGAACATGT
TCTTGGCAATAATTAATGACACCTATTCTGAAGTTAAGGCTGATTATTCAATAGGCAGAA
GACCAGATTTTGAACTTGGTAAAAAATTTCAAAGAGTTGCTTTAATGTTCTCGAGAAAC
TCAGACTCAAGAAAGCTCAAGCTAAAGAAGAAAAAGAAATGCAAACCACTGACTTGGCCC
AGAGAGCCAGAAGAGAAGGCTTTGATGAAAGTGAGATCCAAGAGGCAGAGCAGATGAAAA
GATGGAAGGAAAGGCTTGAAAAAAGTATTATTCTACAGAAATTCAAGACGATTATCAGC
CTGTCACTCAGCAAGAATTCCGAGAGCTCTTTTATACGCGGTGGAGCTTGAGAAGGAAT
TACACTATGTGAGTTTAAAACTGAACCAACTGATGAGAAAGCTGCACTAGCAGGCTGACA
AGTGGAGTCATTTTATAAGAAAAGGCAACCGAAGAATTTCAATTCAGTATGAAGATTTTTT
CCTCTCAATTTTCTTCAGTAATGCAAAAAGAAAAACCAAAAAGTAACCAGAAGTGCTTTTT
ATTTCAAAGTTCTTGAAGTAAAAAGAGTAAAACTCTTGTCCTTTGCTAACAGCCGTGTCTG
CAGTAAAAAATGAAGGAGCCTGCGTGTTCCTAAGTGTGGAGAGGATCTGCGGGAATGT
GGAACAGCTTTCCTTGCTACTGGAACCAACAAGCACACAATGGGACTCTCTGAGTG
CCTGACAAAGTGAACGCAAGTACAGCCAAGCACACATGGTGAAGTGTGAGGGAACACAAG
CACTTTATGGCGTCAACTTTCAAGGAACATATTTTATATGGATTTTGAAGAGTCTTGTTT
GCTGATAAGAACTTCAAGAAGTCTAAGCTTGGCTTTGATTCTCTTGATTCTCTTATATTC
CTCAAGCACCGGAACACGATCCTCCTTCTGGGCATTCTAGGGAAGATAAACTCTGTAA
AGCAAAAAAAGAAAAAAGAAAAA

FIGURE 3